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(71) Applicant *(for all designated States except US)*: THE ROCKEFELLER UNIVERSITY [US/US]; 1230 York Avenue, New York, NY 10021 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : CHAIT, Brian, T. [ZA/US]; 500 East 63rd Street, Apt. 20D, New York, NY 10021 (US). BEAVIS, Ronald [CA/CA]; 55 Pine Bud Avenue, St. John's, Newfoundland A1B 3S7 (CA). WANG, Rong [CN/US]; 500 East 63rd Street, Apt. 26E, New York, NY 10021 (US). KENT, Stephen, B., H. [NZ/US]; 2766 Costebell Drive, LaJolla, CA 92037 (US).

(74) Agent: BURKE, Henry, T.; Wyatt, Gerber, Burke and Badie, 645 Madison Avenue, 5th FLoor, New York, NY 10022 (US).

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(54) Title: METHOD AND PRODUCT FOR THE SEQUENCE DETERMINATION OF PEPTIDES USING A MASS SPECTROMETER

(57) Abstract

Method is described for sequencing polypeptides by forming peptide ladders comprising a series of polypeptides in which adjacent members of the series vary by one amino acid residue and determining the identity and position of each amino acid in the polypeptide by mass spectroscopy.

EXHIBIT 1

METHOD AND PRODUCT FOR THE SEQUENCE DETERMINATION OF PEPTIDES USING A MASS SPECTROMETER

RELATED APPLICATION

This application is a continuation in part of copending and commonly owned application serial number 07/891,177 filed May 29, 1992.

FIELD OF THE INVENTION

This invention relates to rapid and efficient methods for sequencing formed or forming polypeptides utilizing a mass spectrometer.

Polypeptides are a class of compounds composed of α-amino acid residues chemically bonded together by amide linkages with elimination of water between the carboxy group of one amino acid and the amino group of another amino acid. A polypeptide is thus a polymer of α-amino acid residues which may contain a large number of such residues. Peptides are similar to polypeptides, except that they are comprised of a lesser number of α-amino acids. There is no clear-cut distinction between polypeptides and peptides. For convenience, in this disclosure and claims, the term "polypeptide" will be used to refer generally to peptides and polypeptides.

Recent advances in the art of mass spectroscopy have made it possible to obtain characterizing data from extremely small amounts of polypeptide samples. It is, for example, presently possible because of the sensitivity and precision of available instruments to obtain useful data utilizing from picomole to subpicomole amounts of products to be analyzed. Further, the incipient ion-trap technologies promise even better sensitivities, and have already been demonstrated to yield useful spectra in the 10^{-15} to 10^{-16} sample range.

In general, both electrospray and matrix-assisted laser desorption ionizaton methods mainly generate intact molecular ions. The resolution of the electrospray quadrupole instruments is about 1 in 2,000 and that of the laser desorption time-of-flight instruments about 1 in 400. Both techniques give mass accuracies of about 1 in 10-20,000 (i.e. +/- 0.01% or better). There are proposed modifications of time-of-flight analyzer that may improve the resolution by up to factor of 10-fold, and markedly improve the sensitivity of that technique.

reference to the use of a mixture of specific coupling and terminating reagents in the same reaction zone, it will be apparent that the process is equally applicable to the other processes described above.

The system is, of course, applicable to the use of only one disc for the sequencing of a polypeptide or polypeptide mixture.

Although the discs are shown separately on the support, they may also be stacked or replaced with a column of suitably absorbent packing materials.

Further, there may be a number of support members in one device and the chemicals fed to the separate support members through a manifold system so that instead of only one reaction zone, there may be a plurality of reaction zones to still further increase the number of polypeptides which can be simultaneously sequenced.

An especially important embodiment of this invention is that it provides a method of locating covalent modifications on a polypeptide chain particularly post translational modifications of biologically important products which on chemical or

enzymatic hydrolysis produce polypeptides which are phosphorylated, aceylated, glycosylated, cross-linked by disulfide bonds or otherwise modified. Such polypeptides are referred to in this specification and claims as "modified polypeptides".

The inability to directly identify, locate, and quantify modified amino acid residues such as phosphorylated residues in a modified polypeptide is a major shortcoming of standard sequencing methods, and has imposed major limitations on currently important areas of biological research, such as mechanisms of signal transduction. The process of this invention has general application to the direct identification of post-translation modifications present in a peptide chain being sequenced. A modified amino acid residue that is stable to the conditions used in generating the peptide ladder from a formed peptide reveals itself as an additional mass difference at the site of the covalent modification. As described above, from the mass difference, both the position in the amino acid sequence and the mass of the modified amino acid can be determined. The data generated can provide unambiguous identification of the chemical nature of the post translational modification.